

IN THE SPECIFICATION:

Please delete the subheading on page 1, and replace with the following:

~~DESCRIPTION OF THE STATE OF THE ART~~

~~--FIELD OF INVENTION--~~

Page 1, after subheading, please insert the following:

This application pertains to peptides that have antagonistic activity in preventing TGF β 1 from interacting with TGF β 1 receptors.

Page 6, after the last paragraph, please insert the following:

SUMMARY OF INVENTION

Peptides that are antagonists of the binding of TGF β 1 to its receptors in the body. The peptides are characterized in that they have partial amino acid sequences that are identical or similar to those of TGF β 1 itself and/or its receptors.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1. Inhibition of binding of TGF β 1 to the MV-1-Lu cells by peptide P144, measured by flow cytometry. A, image obtained on examining the cells incubated with biotinylated TGF β 1 and developed with avidin-FITC. B, image obtained on examining the cells incubated with avidin-FITC without prior addition of TGF β 1. C, image obtained on examining the cells incubated with TGF β 1 previously incubated with peptide P144 at a concentration of 0.42 μ g/ μ l, and developed with avidin-FITC. The fluorescence emitted is shown on the abscissa, while the ordinate shows the number of cells for each value of fluorescence. The fields corresponding to the cells labelled with TGF β 1-biotin and avidin-FITC (M2) and to the unlabelled cells (M1) are also shown.

Fig. 2. Schematic representation of the process of cirrhosis by CCl₄. Black arrows indicate when two weekly doses of CCl₄ were administered to the rats, and black dashed arrows show when there was one weekly dose. The grey arrows indicate administration of peptide P144. A: Healthy controls; B: Healthy controls + P144, B₁: with peptide 70 μ g/day; C: Cirrhotic; C₁ with saline; C₂ with peptide 70 μ g/day; D: Cirrhotic with CCl₄ + phenobarbital; D₁ plus saline; D₂ plus peptide 70 μ g/day.

Fig. 3. Effect of TGF β 1 on growth of MV-1-Lu cells. The cells are cultivated at a density of 5000 cells/well at

the concentrations of TGF β 1 indicated, pg/ml Abscissa:
TGF β 1 concentration (pg/ml); Ordinate: c.p.m.

Fig. 4. Percentage inhibition of TGF β 1 (200 pg/ml) by peptides from TGF β 1. All the peptides were tested at a concentration of 200 μ g/ml. Inhibition of TGF β 1 of 100% corresponds to the growth of MV-1-Lu cells that is obtained in the absence of TGF β 1.

Fig. 5. Percentage inhibition of the activity of TGF β 1 (200 pg/ml) in the presence of various nominal concentrations of peptide P12, filtered (\blacklozenge) and unfiltered (\bullet).

Fig. 6. Percentage inhibition of TGF β 1 (200 pg/ml) by peptides from TGF β 1. All the peptides were tested at a concentration of 200 μ g/ml. Inhibition of TGF β 1 of 100% corresponds to the growth of MV-1-Lu cells that is obtained in the absence of TGF β 1.

Fig. 7. Autoradiograph of an affinity labelling test of the receptors of TGF β 1. Lane C1: effect of incubation of the cells with a concentration of 0.16 μ M of 125 I-TGF β 1 which corresponds to an activity of 0.3 μ Ci (positive control). Lane C2: effect of preincubation of the cells with a concentration of non-radioactive TGF β 1 10 times greater than that of 125 I-TGF β 1 (negative control). Lane C3: preincubation was effected with peptide P29 at a concentration 10^6 times greater than the molar concentration of 125 I-TGF β 1. It can be seen that there is inhibition of the binding of 125 I-TGF β 1 to the type I, II and III cell receptors both by peptide P29 and by non-radioactive TGF β 1.

Fig. 8. Autoradiograph of an affinity labelling test of the receptors of TGF β 1. Lanes C1 to C6: effect of preincubation of the MV-1-Lu cells, with different concentrations of peptide P29 (10^6 , 8×10^5 , 6×10^5 , 4×10^5 , 2×10^5 and 10^5 times the molar concentration of ^{125}I -TGF β 1 respectively), prior to addition of ^{125}I -TGF β 1. Lane C7: effect of preincubation of the MV-1-Lu cells with unlabelled TGF β 1 (10^2 times the molar concentration of ^{125}I -TGF β 1) prior to addition of ^{125}I -TGF β 1 (negative control). Lane C8: effect of incubation of the MV-1-Lu cells with a concentration of $0.42 \mu\text{M}$ of ^{125}I -TGF β 1, corresponding to an activity of $0.4 \mu\text{Ci}$, without prior preincubation (positive control).

Fig. 9. Percentage inhibition of TGF β 1 (200 pg/ml) by receptor peptides predicted as complementary to regions of TGF β 1. All the peptides were tested at a concentration of $200 \mu\text{g/ml}$. Inhibition of TGF β 1 of 100% corresponds to the growth of MV-1-Lu cells that is obtained in the absence of TGF β 1.

Fig. 10. Percentage inhibition of TGF β 1 (200 pg/ml) by overlapping peptides derived from the extracellular region of the type III receptor. All the peptides were tested at a concentration of $200 \mu\text{g/ml}$. Inhibition of TGF β 1 of 100% corresponds to the growth of MV-1-Lu cells that is obtained in the absence of TGF β 1.

Fig. 11. Percentage inhibition of TGF β 1 (200 pg/ml) by overlapping peptides derived from the extracellular region of the type III receptor. All the peptides were tested at a concentration of $200 \mu\text{g/ml}$. Inhibition of TGF β 1 of 100% corresponds to the growth of MV-1-Lu cells that is obtained in the absence of TGF β 1.

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Fig. 13. Percentage inhibition of the activity of TGF β 1 (200 pg/ml) in the presence of different nominal concentrations of peptide P54, filtered (\blacklozenge) and unfiltered (\bullet).

Fig. 14. Percentage inhibition of TGF β 1 (200 pg/ml) by receptor peptides derived from modification of peptide P54 (P139 to P143) and of the peptides derived from the human type III receptor (P144 and P145). All the peptides were tested at a concentration of 200 μ g/ml. Inhibition of TGF β 1 of 100% corresponds to the growth of MV-1-Lu cells that is obtained in the absence of TGF β 1.

Fig. 15. Percentage inhibition of the activity of TGF β 1 (200 pg/ml) in the presence of different nominal concentrations of peptide P144 without filtration.

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to the cell receptors both by peptide P144 and by the non-radioactive TGF β 1.

Fig. 17. Percentage inhibition of TGF β 1 (200 pg/ml) by peptides derived from human type II receptor (P146), from fetuin (P147 to P149) and from endoglin (P150 to P154). All the peptides were tested at a concentration of 200 μ g/ml. Inhibition of TGF β 1 of 100% corresponds to the growth of MV-1-Lu cells that is obtained in the absence of TGF β 1.

Fig. 18. Percentage inhibition of TGF β 1 (200 pg/ml) by peptides derived from α 2-macroglobulin. All the peptides were tested at a concentration of 200 μ g/ml. Inhibition of TGF β 1 of 100% corresponds to the growth of MV-1-Lu cells that is obtained in the absence of TGF β 1.

Fig. 19. Percentage inhibition of the binding of TGF β 1 to MV-1-Lu cells by various synthetic peptides. Inhibition was investigated by measuring the percentage of labelled cells (emit fluorescence) and unlabelled cells (do not emit fluorescence) for each peptide (Material and Methods).

Fig. 20. Effect of administration of peptide P144 on collagen synthesis during experimental cirrhosis induction with CCl₄. The ratio of collagen to total protein is shown on the ordinate. The abscissa shows the various groups of rats: Co= healthy rats; Co+P144= healthy rats treated with peptide P144; Tto₁= rats subjected to induction of cirrhosis with CCl₄ and administered peptide P144 on alternate days during this period and Ci₁= rats subjected to induction of cirrhosis with CCl₄ for 11 weeks and not treated with peptide P144.

Fig. 21. Effect of administration of peptide P144 on collagen synthesis during experimental cirrhosis induction with CCl_4 . The ordinate shows the ratio of the area of fibrosis to the total area in tissue preparations stained with Sirius Red. The abscissa shows the various groups of rats: Co= healthy rats; Co+P144= healthy rats treated with the peptide; Tto₁= rats subjected to induction of cirrhosis with CCl_4 and administered peptide P144 on alternate days during this period and Ci₁= rats subjected to induction of cirrhosis with CCl_4 for 11 weeks and not treated with peptide P144.

Fig. 22. Effect of administration of peptide P144 on collagen synthesis once cirrhosis has been induced with CCl_4 . The ordinate shows the ratio of collagen to total protein. The abscissa shows the various groups of rats: Co= healthy rats; Co+P144= healthy rats treated with the peptide; Tto₂= rats subjected to induction of cirrhosis with CCl_4 and administered peptide P144 on alternate days at the end of this period and Ci₂= rats subjected to induction of cirrhosis with CCl_4 for 11 weeks and not treated with peptide P144.

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Fig. 24. Comparison of the data on quantity of collagen and area of fibrosis, obtained by the two techniques employed. The abscissa shows the values of the ratio of the area of fibrosis to the total area, obtained by image analysis. The ordinate shows the values of the ratio of μg of collagen to mg of total protein, obtained by spectrophotometric analysis of liver sections stained with Direct Red and Fast Green. R^2 is shown. ($F \leq 0.001$).

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Page 7, please delete the subheading and replace with the following:

~~DETAILED DESCRIPTION OF THE INVENTION~~

-DETAILED DESCRIPTION--

Please delete pages 40 - 47 starting with the subheading "DESCRIPTION OF THE FIGURES".

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